Effect of Hypophysectomy on the Incorporation of Proline in the Collagen Fractions[1]

Although hypophysectomy is known to influence the content[2] and the biosynthesis[3–5] of collagen in experimental animals, the mechanism has not been investigated in more detail.

The rats were hypophysectomized by the method of SATO and YONEDA[6] at the age of 1 month (weight 48–53 g), 2–3 weeks before the experiments. The control animals included normal rats of the same weight and of the same age. All the data are averages of 2 rats, which differed by less than 5%. Porcine somatotrophin (STH) or L-thyroxine were injected to some of the hypophysectomized animals for 4 days. The control animals received the same volume of solvent (0.15 *M* NaCl). The single dose of [^{3}H]proline-G (0.5 μCi/g, The Radiochemical Centre, Amersham, England) was injected i.p., simultaneously with the first injection of the hormones.

During the 84-h experimental period, the average weight gains were in the hypophysectomized rats 0.5 g (initial weight 56 g), in the normal rats of the same weight 12.5 g and in the normal rats of the same age 16.5 g (initial

[1] Supported by institutional grants from the Sigrid Jusélius Foundation and U.S. Department of Agriculture, Foreign Research and Technical Programs Division.

[2] R. O. Scow and S. N. HAGAN, Endocrinology *77*, 852 (1965).

[3] K. KOWALEWSKI, Acta endocr., Copenh. *50*, 321 (1965).

[4] T. M. CHULKOVA and V. N. OREKHOVITCH, Vop. med. Khim. *11*, 76 (1965); cited according to Collagen Currents *6*, 426 (1966).

[5] W. H. DAUGHADAY and I. K. MARIZ, J. Lab. clin. Med. *59*, 741 (1962).

[6] M. SATO and S. YONEDA, Acta endocr., Copenh. *51*, 43 (1966).

weight 140 g). The administration of STH to hypophysectomized rats corrected the weight gain to 13.0 g, but thyroxine increased it by 2.5 g only.

Immediately after killing, 5 g of cleaned skin was homogenized. Collagen fractions soluble into 0.15 *M* NaCl, 0.45 *M* NaCl and 0.5 *M* acetic acid and the insoluble collagen were prepared[7]. The 0.15 *M* NaCl-soluble supernatant was precipitated with four-fold volume of ice-cold ethanol, centrifuged at 35,000 *g* for 1 h and the filtered supernatant analyzed on free hydroxyproline and total hydroxyproline. The difference was accepted as peptide-bound hydroxyproline[8].

Aliquots of the soluble fractions of collagen and the whole insoluble residue were hydrolyzed at 130 °C for 3 h in 6 *N* hydrochloric acid. The contents of hydroxyproline[9] and its radioactivities[10] were determined.

The hypophysectomy reduced the more soluble, i.e., younger forms of collagen (Figure 1) and very markedly decreased the incorporation of proline to collagen hydroxyproline (Figure 2). Although the administration of STH restored the weight increase in the hypophysectomized rats, the same effect is only slightly seen in the incorporation of proline to collagen hydroxyproline, probably because of the excretion of labelled proline before the action of the hormone. However, STH and, to less extent, thyroxine increased the incorporation of proline into salt-soluble collagen fractions. The distribution of the label in the fractions suggests that the synthesis of soluble collagen is affected more than its maturation to acid-soluble or insoluble collagen. The effect of thyroxine is in agreement with the earlier results from our laboratory[11].

The concentration of ethanol-soluble hydroxyproline in the skins of the normal rats was about two-fold in comparison with the hypophysectomized rats.

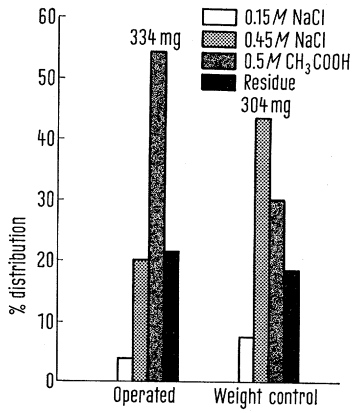

Fig. 1. Distribution of the indicated collagen fractions in hypophysectomized and control rats of the same weight.

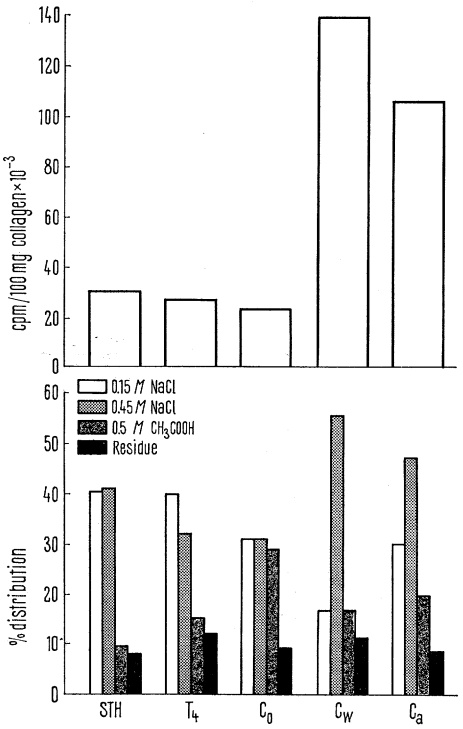

Fig. 2. Top: total hydroxyproline activity in skin samples. Below: distribution of hydroxyproline activity between the indicated collagen fractions. C_o, hypophysectomized rats; C_w, control animals of the same weight; C_a, control animals of the same age; T_4, hypophysectomized rats treated with thyroxine (Lääke Oy, Turku, Finland; 0.6 µg/g/day); STH, hypophysectomized rats treated with somatotrophin (Somacton, Ferring AB, Malmö, Sweden; 2 µg (0.002 I.U.) per g/day).

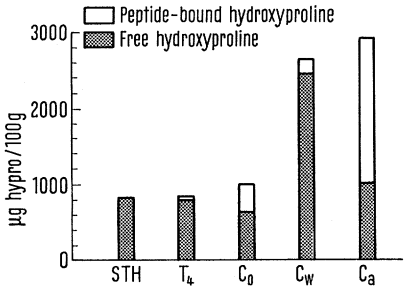

Fig. 3. Peptide-bound and free hydroxyproline in the ethanol-soluble fraction of rat skin. The designations are explained in the legend of Figure 2.

Zusammenfassung. In hypophysektomierten, wachsenden Ratten ist die Biosynthese des löslichen Kollagens schwer gehemmt. Die Reifung zum unlöslichen Kollagen ist weniger beeinflusst.

M. Valavaara, E. Heikkinen and E. Kulonen

Department of Medical Chemistry, University of Turku, Turku (Finland),
1 March 1968.

[7] E. Heikkinen and E. Kulonen, Acta physiol. scand. *68*, 231 (1966).
[8] K. I. Kivirikko, Acta physiol. scand. *60*, 32 (1963).
[9] J. F. Woessner jr., Archs Biochem. Biophys. *93*, 440 (1961).
[10] K. Juva and D. J. Prockop, Analyt. Biochem. *15*, 77 (1966).
[11] L. Mikkonen, K. Lampiaho and E. Kulonen, Acta endocr., Copenh. *51*, 23 (1966).